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Advanced bioprinting strategies using decellularized matrix as a substrate to promote bone regeneration

Running title: dECM bioprinting for bone regeneration

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Abstract

The central challenge in bone tissue engineering is not merely to fabricate bone-like materials, but to reconstruct a regenerative microenvironment that recapitulates native bone. Decellularized extracellular matrix (dECM) retains tissue-specific structural components and biological cues and is therefore regarded as a promising biomaterial for bone regeneration. However, its poor mechanical strength, structural instability, and limited printability restrict its application in complex bone defects. Recent advances in bioprinting, together with chemical modification, bioactive substance incorporation, and biomimetic structural design, have provided new opportunities to improve the osteogenic and functional properties of dECM-based constructs. This review first outlines the major biological processes involved in bone repair and introduces the principal bioprinting technologies and dECM sources relevant to bone tissue engineering. It then discusses recent strategies for optimising dECM sources and processing methods, modifying material properties, incorporating bioactive substances, and constructing biomimetic architectures. Particular attention is given to how these approaches promote osteogenesis, vascularisation, and functional bone reconstruction. Current limitations and future directions for clinical translation are also considered. By integrating bone repair processes, dECM characteristics, bioprinting technologies, and functional enhancement strategies, this review clarifies how advanced bioprinting can improve the regenerative performance of dECM and guide the development of next-generation functional materials for bone defect repair.

Keywords: Decellularized extracellular matrix; Bioprinting; Bone remodeling

1. Introduction

Bone tissue inherently possesses a certain regenerative potential; however, when bone defects are severe and exceed the body's capacity for self-healing, the body's own repair mechanisms alone are often insufficient to restore normal tissue structure and mechanical function¹. Clinically, issues such as large bone defects, complex trauma, bone infections, reconstruction following bone tumor resection, and poor bone healing after spinal fusion surgery remain widespread² and continue to impact patients' functional recovery and quality of life. Autologous bone grafting has long been regarded as a key method for repairing bone defects; however, its further application has been limited by issues such as limited bone supply, donor site complications, and additional surgical trauma. Although allograft bone and synthetic bone substitutes have alleviated the shortage of bone sources to some extent, they still struggle to simultaneously meet the multifaceted requirements of bioactivity, tissue integration, and long-term remodeling.

In recent years, the rapid advancement of 3D bioprinting technology has provided new solutions for the construction of complex bone tissues. Compared with traditional scaffold preparation methods, bioprinting enables the precise spatial distribution of materials, cells, and bioactive factors, and allows for the construction of personalized tissue-engineered scaffolds based on patient imaging data, thereby better simulating the compositional and structural characteristics of natural bone tissue³. Currently, bioprinting has been widely applied in fields such as bone, cartilage, the bone-cartilage interface, and spinal tissue engineering, and shows great promise. However, most existing bioprinting materials are based on natural polymers, bioceramics, or synthetic polymers. Although they can provide a certain degree of mechanical support or printability, they struggle to fully replicate the complex extracellular matrix microenvironment of natural bone tissue, which remains one of the key factors limiting their further development⁴.

The extracellular matrix (ECM) is a crucial component of the tissue microenvironment; it not only provides structural support but also plays a role in regulating various biological processes, such as cell adhesion, migration, proliferation, and differentiation⁵. Decellularized extracellular matrix (dECM), created by removing the cellular components from tissue while retaining collagen, glycosaminoglycans, adhesion molecules, and some tissue-derived biological signals, is therefore considered an important bridge between natural tissues and artificial materials⁶. Compared with traditional natural hydrogels, the most distinctive feature of dECM is its tissue specificity. dECMs

from different sources often retain distinct microenvironmental characteristics; for example, bone-derived dECM exhibits stronger osteogenic induction potential, cartilage-derived dECM is more conducive to maintaining the cartilaginous phenotype, while muscle- and fat-derived dECMs demonstrate unique advantages in vascularization and tissue reconstruction⁷. This biological information inherent in natural sources is difficult for most synthetic materials to replicate, and it is a key reason why dECM has garnered widespread attention in recent years.

Bone repair is inherently a dynamic process that typically progresses through three consecutive stages: the inflammatory response, tissue repair, and bone remodeling⁸. Each stage corresponds to distinct cellular activities and microenvironmental requirements: the early stage requires moderate immune regulation; the middle stage relies on angiogenesis and the formation of new tissue; and the late stage involves a long-term balance between bone formation and resorption⁹. Therefore, an ideal bone repair material must not only provide mechanical support but also be capable of participating in and regulating this complex regenerative process. From this perspective, the natural microenvironmental advantages retained by dECM make it a key candidate for the development of functional bone repair materials.

Although dECM has a solid biological foundation, its practical application still faces a series of challenges. Natural dECM often exhibits inadequate mechanical properties and limited shaping accuracy and structural integrity; its performance is also susceptible to variations in tissue source, decellularization, and digestion processes, which is particularly evident in load-bearing bone tissue repair¹⁰. Relying solely on dECM often makes it difficult to obtain ideal constructs that balance biological activity and structural performance¹¹. In recent years, advances in bioprinting technology have provided new solutions to this problem. By precisely controlling material deposition methods and spatial structures, bioprinting enables the customized construction of complex tissues and provides a platform for the integration of cells, bioactive factors, and various functional components¹². At the same time, strategies such as chemical modification, bioactive substance loading, composite material construction, and biomimetic structural design for dECM have continued to emerge, gradually transforming it from a simple natural material into a multifunctional engineered bio-ink system.

Currently, research on dECM bioprinting is no longer limited to reproducing specific morphologies or spatial structures, but has gradually expanded to the design of functional

constructs that integrate mechanical support, osteogenic induction, vascularization, and tissue remodeling^{13,14}. To address the issues of insufficient printability and structural stability in dECM, researchers have attempted to synergistically optimize its material properties and regenerative functions through approaches such as composite construction, cross-linking or chemical modification, delivery of bioactive substances, and biomimetic structural design^{11,13,15}. These strategies have enabled dECM to evolve from a matrix material that merely mimics the components of natural tissues into an engineered bio-ink system capable of regulating the microenvironment for bone repair.(Figure 1)

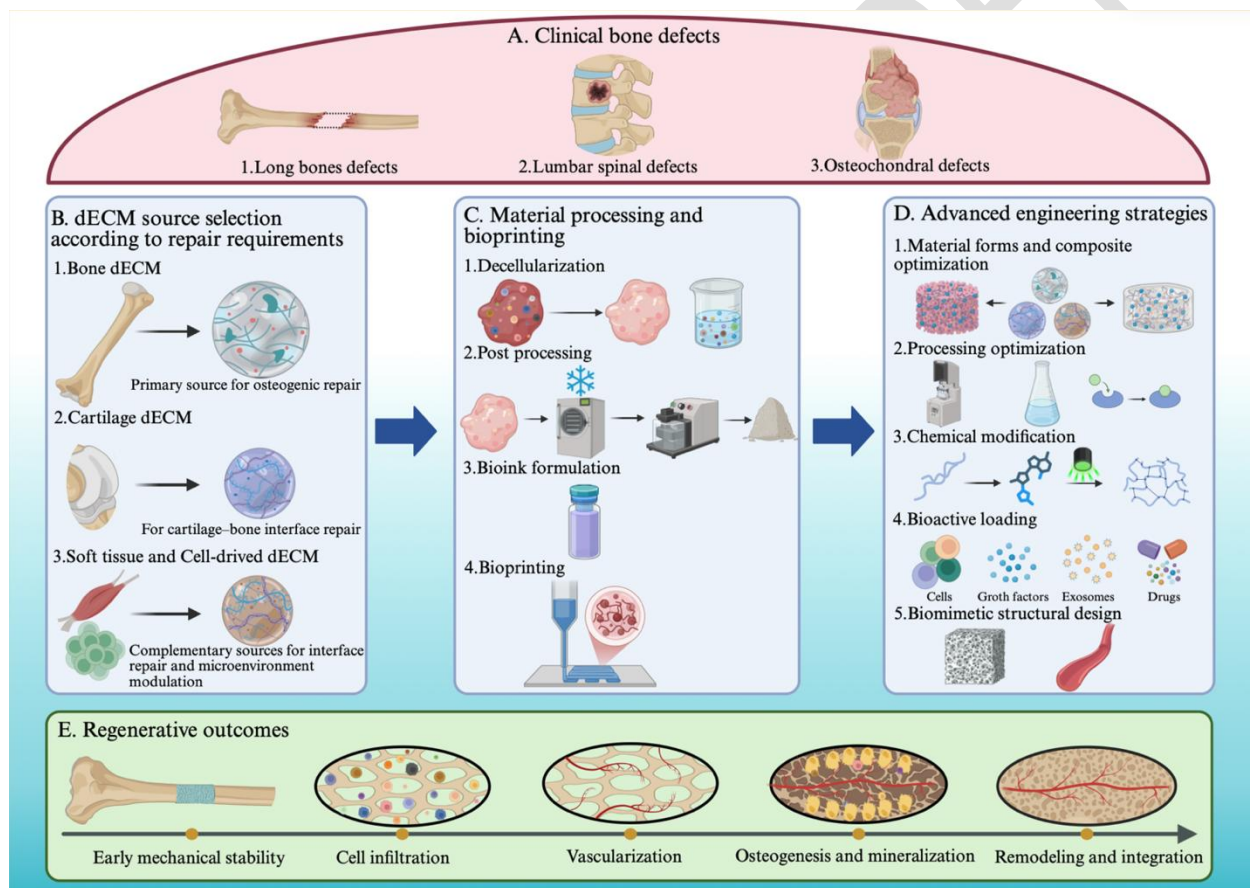


Figure 1. A comprehensive framework for bone regeneration bioprinting strategies based on advanced decellularized extracellular matrix (dECM). (A) Typical clinical scenarios requiring bone tissue reconstruction, including long-segment femoral defects, vertebral fusion for spinal metastases, and articular osteochondral defects. (B) Major extracellular matrix sources used for regenerative construct design, including bone, cartilage, soft tissue, and cells. (C) A general workflow for bioprinting based on decellularized extracellular matrix (dECM), including tissue decellularization—removing cellular components while preserving extracellular matrix components—followed by post-processing via washing, freeze-drying, and grinding; dissolution or digestion; neutralization; and bio-ink formulation; and finally, multi-material bioprinting via

extrusion or other methods. (D) Five key engineering strategies for improving dECM-based constructs: material and composite optimization, process optimization, chemical modification, loading of bioactive components, and biomimetic structural design. (E) Desired regenerative outcomes during bone healing, including early structural stability, cellular infiltration, angiogenesis, osteogenesis and mineralization, as well as long-term tissue remodeling and host integration.

However, for bone repair applications, there is still a lack of a unified design and evaluation framework for establishing appropriate matches between dECMs from different tissue sources, processing methods, and functional enhancement strategies based on the location of the defect, load-bearing characteristics, and regenerative requirements. This also poses certain challenges for cross-comparisons among different studies, material standardization, and subsequent clinical translation.

Therefore, this paper provides a review of the application of decellularized matrices in bone tissue engineering. It begins by outlining the bone repair process and its biological basis, then summarizes bioprinting technologies applicable to dECM and the characteristics of dECM from different sources. It focuses on advanced dECM-based bioprinting strategies, including research progress in material source optimization, processing methods, chemical modification, loading of bioactive substances, and the construction of biomimetic structures. Finally, the paper analyzes current key challenges and future development directions, with the aim of providing guidance for the design and clinical translation of a new generation of functionalized bone repair materials.

2. The Three Stages of Bone Repair

Bone repair is not simply a matter of “growing new bone” at the site of a defect, but rather a continuous process in which the inflammatory response, tissue regeneration, and long-term remodeling are interconnected. For the sake of description, clinical and experimental studies typically categorize this process into the inflammatory phase, the repair phase, and the bone remodeling phase; however, these stages are not strictly separated in time but rather overlap significantly¹⁶. The repair pattern of bone defects is also jointly influenced by local stability, blood supply conditions, and the defect microenvironment. In situations where relative stability is insufficient, repair is often dominated by secondary healing, which may involve processes such as hematoma formation, granulation and fibrous tissue formation, chondroblastic tissue formation, and callus mineralization, accompanied by endochondral and intramembranous ossification; whereas under conditions of anatomical reduction and absolute stability, direct bone healing may

occur, typically without the formation of a distinct callus¹⁶. For dECM bioprinted constructs, understanding the primary tasks at each stage is particularly important, as the material's degradation behavior, pore connectivity, mechanical support, and bioactive design must be tailored to the phasic requirements of local tissue repair, rather than functioning in the same manner throughout the entire repair process¹⁷.

2.1 Inflammatory Phase

Following an injury, local vascular rupture and bleeding can rapidly lead to the formation of a hematoma. A hematoma is not merely a residual product of the injury, but rather the earliest temporary microenvironment formed during the bone repair process. The fibrin network within the hematoma, along with mediators released by platelets and local cytokines, provides the initial conditions for the recruitment, adhesion, and subsequent migration of inflammatory cells and mesenchymal progenitor cells¹⁸. Neutrophils typically enter the injury site early on, followed by monocytes and macrophages, which further participate in the clearance of necrotic tissue and cellular debris; in infection-related injuries, these cells also participate in the clearance of local pathogens and jointly regulate the early inflammatory response¹⁹.

The key during the inflammatory phase is not to “eliminate inflammation” as much as possible, but rather to establish an inflammatory response of appropriate intensity that resolves in a timely manner. Excessive suppression of early inflammation may hinder local clearance and cell recruitment¹⁸; conversely, if inflammation continues to intensify or fails to resolve promptly, it may interfere with angiogenesis and osteogenesis, increasing the risk of delayed healing or non-union¹⁹. In other words, what is needed in the early stages of bone repair is not a completely quiescent local environment, but rather a microenvironment capable of facilitating clearance, cell recruitment, and the transition to regenerative signaling.

Immunocompatibility is a fundamental requirement for dECM applications, and thorough decellularization is a key prerequisite for reducing the risk of immune rejection. Regardless of the stage of tissue repair, residual cells and potentially immunogenic components must be removed. During the inflammatory phase, it is also necessary to optimize the material composition, cross-linking method, and microstructure to reduce nonspecific inflammation and promote cell infiltration and mass exchange^{20,21}. dECM constructs should, during the inflammatory phase, first minimize nonspecific inflammatory responses caused by residual cellular components,

decellularization reagents, or cross-linking-related components, while simultaneously reserving sufficient space for cell infiltration and local substance exchange. The collagen, glycosaminoglycans, and adhesion-related components retained in the dECM provide a foundation for early cell adhesion and migration; however, its immunocompatibility remains influenced by various factors, including tissue origin, decellularization efficiency, residual DNA and cellular debris, sterilization methods, and subsequent cross-linking treatments²⁰. Therefore, when evaluating dECM bio-inks, in addition to cell viability and printed morphology, attention should also be paid to inflammatory cell infiltration in the early post-implantation period, the macrophage response, and their relationship to subsequent tissue integration.

2.2 Repair Phase

As acute inflammation gradually subsides, bone repair enters a phase characterized primarily by progenitor cell recruitment, angiogenesis, and the formation of new tissue. Under the combined influence of local oxygen tension, microenvironmental signals, and mechanical conditions, mesenchymal progenitor cells can differentiate into osteogenic or chondrogenic cell lineages¹⁶. In typical secondary bone healing, fibrous and chondroplastic tissues often form first in the defect site, followed by the formation of a cartilaginous callus, which gradually transforms into a bony callus after mineralization; under conditions of high stability and narrow tissue gaps, repair may rely more on intramembranous ossification and result in reduced formation of the cartilaginous intermediate stage²².

dECM not only serves as a physical scaffold for cell growth but also provides biological cues for progenitor cell recruitment and fate regulation by retaining collagen, glycosaminoglycans, adhesion molecules, and various matrix-associated signaling molecules found in the native extracellular matrix. Certain dECMs can promote the adhesion, migration, and local retention of mesenchymal progenitor cells through their matrix components and the chemokines bound to them, thereby increasing the cellular supply to defect sites²³. At the same time, the tissue-specific composition of dECM and its mechanical microenvironment can jointly regulate cell adhesion, cytoskeletal remodeling, and related signaling pathways, thereby promoting the differentiation of mesenchymal stem/progenitor cells toward the osteogenic lineage. Recent studies have shown that dECM from different sources and prepared under different conditions exhibits varying osteogenic induction capabilities. For example, bone-

derived dECM can enhance the expression of osteogenesis-related genes and mineralization in mesenchymal stromal cells, while compositional changes in cell-derived dECM can also influence osteogenic differentiation through signaling pathways such as FAK/PI3K/AKT²⁴. Therefore, the role of dECM in bone repair is not limited to providing a scaffold for cell adhesion; rather, it is capable of synergistically regulating cell recruitment, proliferation, and osteogenic differentiation through its retained biochemical signals and physical microenvironment.

Angiogenesis occurs throughout the entire repair period and is closely coupled with the osteogenesis process. New blood vessels not only supply oxygen and nutrients but also create the necessary conditions for progenitor cell migration, the removal of metabolic waste, and new bone formation²⁵. For large bone defects, the central region is distant from the peripheral blood supply, and relying solely on peripheral vascular ingrowth often fails to meet regenerative needs. Therefore, insufficient vascularization remains one of the key limitations that bone tissue engineering constructs must address when transitioning from small-scale animal models to applications for large-volume, weight-bearing bone defects^{22,26}.

Given the potential regulatory role of the dECM in cell recruitment and osteogenic differentiation, this stage imposes more specific requirements on dECM bioprinted constructs: the material must retain biological cues that promote cell adhesion, migration, and osteogenic differentiation, while also allowing for vascular ingrowth and maintaining the basic spatial structure until new bone is formed. At this stage, more specific requirements are placed on dECM bioprinted constructs: the material must allow for cell migration and vascular ingrowth while maintaining the basic spatial structure until new bone has formed. Pure dECM hydrogels typically exhibit good cell compatibility and tissue-relevant bioactivity but offer limited mechanical support; therefore, they are often combined with bioceramics, synthetic polymers, or cross-linked systems to balance structural stability and regenerative function²⁷. In practical design, bone-derived dECM can be used in synergy with mineralization-related components for the repair of bony defects; for the reconstruction of the osteochondral interface or subchondral bone, cartilage-derived dECM can serve as part of a tissue-specific microenvironment; and for defect models requiring rapid establishment of blood supply, greater emphasis should be placed on pore connectivity, vascular

channel design, and the synergistic application of bioactive components that promote angiogenesis^{22,26}.

2.3 Bone Remodeling Phase

Once preliminary mineralized tissue has formed in the defect site, bone repair is not yet complete. In cases where secondary bone healing predominates, early new bone typically consists mainly of woven bone and mineralized bone callus, which can restore the continuity and partial stability of the defect site in the short term; however, its microstructure and mechanical properties still differ from those of mature bone¹⁶. Subsequently, osteoclasts resorb the early-formed bone tissue, while osteoblasts redeposit bone matrix in the corresponding areas; the interaction between these two cell types leads to the gradual replacement and reorganization of the bone tissue. In a repair model dominated by bone callus, the volume of the bone callus gradually decreases, the arrangement of trabeculae and the contour of the cortical bone tend to be restored, ultimately forming lamellar bone with a more ordered structure and mechanical properties closer to those of normal bone²⁸.

The remodeling period is relatively long and is closely related to the local mechanical environment. Appropriate fixation stability and a gradual return to physiological mechanical loading help the newly formed bone adapt to local stress demands; conversely, persistent instability, excessive mobility, or stress shielding caused by high-stiffness implants may alter normal stress transmission and impair bone structural recovery²⁹. Therefore, the evaluation of bone repair materials should not be limited to early-stage mineralization or increases in new bone volume; it should also focus on whether the materials can form an appropriate mechanical interface with the newly formed bone during the mid- and late stages. If the material degrades too rapidly, it may lose its supportive function before the new bone has acquired sufficient load-bearing capacity³⁰; conversely, if the material degrades too slowly or maintains a stiffness significantly higher than that of the surrounding bone tissue over the long term, it may also interfere with local stress distribution and subsequent remodeling³¹.

For dECM bioprinted constructs, the bone remodeling phase places greater emphasis on the dynamic balance among material degradation, mineralization processes, and mechanical support³².

While dECM can provide certain tissue-specific matrix information, it is often difficult to balance long-term structural maintenance and load-bearing requirements when used alone; therefore, optimization requires the integration of composite materials, cross-linking methods, or structural design³³. An ideal construct should not merely aim to induce extensive mineralization in the short term; rather, it should gradually give way to host tissue as new bone forms, while maintaining pore connectivity and a mechanical environment conducive to long-term remodeling³⁴. Existing studies have shown that combining bone-derived dECM with polymers, ceramics, or bioactive components can, to some extent, improve the mechanical properties, osteogenic performance, and *in vivo* osseointegration of the construct³⁵. Therefore, bioprinting strategies for dECM in bone repair should adopt a phased approach: minimizing unnecessary inflammatory damage in the early stage, supporting cellular infiltration, vascularization, and new bone formation in the middle stage, and balancing material degradation with bone tissue remodeling in the late stage^{9,22}. This design logic also forms the foundation for subsequent discussions on dECM source selection, processing methods, chemical modification, and the construction of biomimetic structures.(Figure 2)

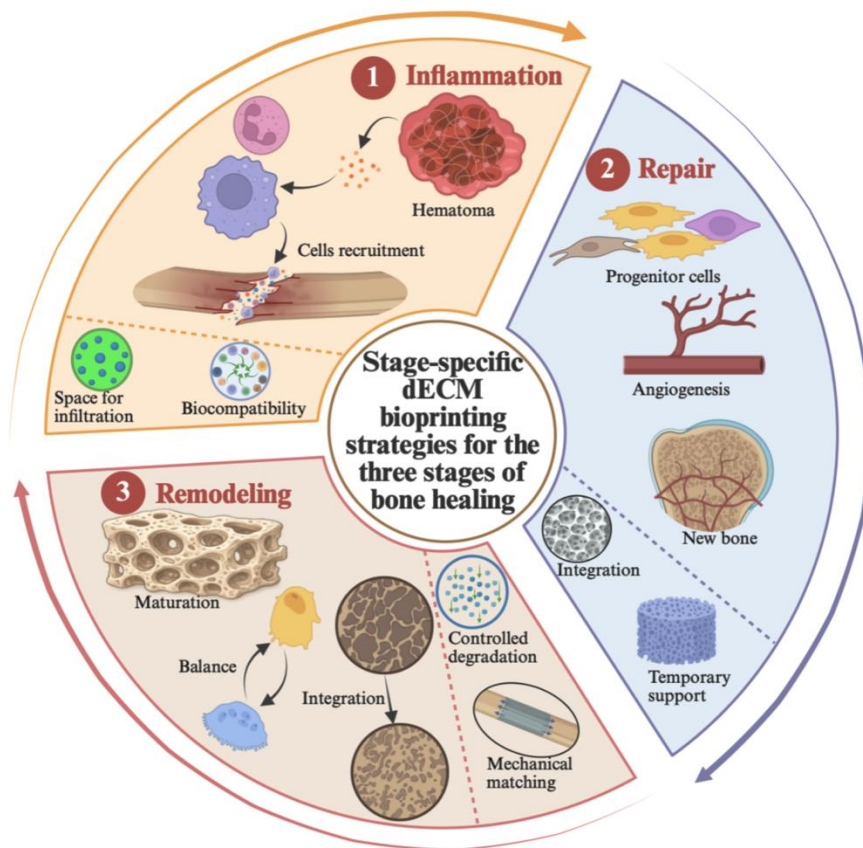


Figure 2. The three stages of bone healing and the stage-specific design requirements for dECM constructs during each stage. (A) The inflammatory phase involves hematoma formation, immune cell infiltration, debris clearance, and progenitor cell recruitment. dECM constructs should minimize immunogenic residues while supporting biocompatibility and providing adequate space for the early inflammatory response. (B) The repair phase is characterized by progenitor cell differentiation, angiogenesis, cartilage or early bone formation, and the development of a mineralized bone callus. The construct should provide a porous structure to facilitate vascular sprouting, a microenvironment conducive to osteogenesis, and temporary structural stability. (C) During the remodeling phase, the activity of osteoblasts and osteoclasts tends toward equilibrium, promoting osseointegration with the host. Material degradation and the loss of structural support from the construct should occur in tandem with the newly formed bone's increasing load-bearing capacity.

3. Bioprinting Technology

Bioprinting involves the layer-by-layer deposition or curing of cells, biomaterials, and necessary bioactive components in predetermined spatial positions, guided by digital models, to construct tissue-engineered constructs that possess both three-dimensional morphology and biological function^{2,36}. Unlike traditional 3D printing, which primarily focuses on material shaping and mechanical properties, bioprinting must also take into account key factors such as cell viability, material cross-linking, structural precision, and post-implantation tissue integration during the manufacturing process³⁷. This distinction is particularly evident in bone repair: repairing bone defects not only requires the construct to provide necessary mechanical support in the early stages but also depends on cellular infiltration, vascularization, and the subsequent ongoing processes of mineralization and bone remodeling. Therefore, the goal of bioprinting is not merely to “print material into the shape of bone,” but to reconstruct a local microenvironment within the defect site that combines spatial structure, a viable cellular environment, and regenerative function^{2,36,37}.

Different printing technologies have their own strengths in terms of fabrication precision, suitable material viscosity, cell compatibility, printing speed, and construct size; therefore, there is no single, absolutely optimal technical approach suitable for all bone defect scenarios. For dECM bio-inks, the choice of printing method is further constrained by their viscoelasticity, cross-linking mechanisms, and ability to maintain structural integrity after printing^{10,38}. Natural dECM hydrogels typically exhibit good biocompatibility and tissue-relevance, but when used alone, they often struggle to simultaneously achieve high printing accuracy, stable shape retention, and long-term mechanical support^{10,38,39}. Therefore, in bone repair applications, a more feasible strategy is typically to combine dECM with other materials, cells, or fabrication methods based on the defect

site and regeneration goals, in order to strike a balance between bioactivity, printability, and structural stability³⁹.

3.1 Extrusion-Based Bioprinting

Extrusion-based bioprinting is currently one of the most widely used technologies in bone tissue engineering. Its basic principle involves using drive mechanisms such as air pressure, pistons, or screws to continuously extrude bio-ink through a nozzle, depositing it layer by layer along a predetermined path to form a three-dimensional structure⁴⁰. Due to its relatively wide adaptability to material viscosity, this technology can print high-concentration hydrogels, cell suspensions, and composite bio-inks, and can construct scaffolds with specific porosity and connectivity through path design⁴¹.

Extrusion-based technology is highly adaptable for dECM bioprinting. The rheological properties of dECM can be improved by adjusting its concentration, temperature, or by blending it with other hydrogels, and its structure can be stabilized post-printing through thermosensitive, ionic, or photochemical cross-linking^{10,11}. This design not only helps preserve the role of dECM as a cellular microenvironment but also improves the shape retention of the printed construct to a certain extent. Previous studies have shown that composites of bone-derived dECM and ceramic components can further enhance the material's bioactivity and osteogenic performance⁴². For bone repair, extrusion-based printing also facilitates the integration of dECM with components such as hydroxyapatite, calcium phosphate, bioglass, or biodegradable polymers to improve the mineralization capacity and mechanical properties of the printed constructs⁴³.

However, the shear stress generated during the extrusion process remains a concern that requires attention. A nozzle diameter that is too small, excessive material viscosity, or excessive printing pressure can all increase the mechanical stress on cells during extrusion, thereby affecting cell viability and phenotype⁴⁴. Therefore, extrusion-based printing should not simply aim for higher viscosity or greater precision, but rather seek a reasonable balance among printability, structural stability, and cell tolerance⁴⁵. For load-bearing or large-volume bone defects, extrusion-based bioprinting is generally better suited as part of a hybrid manufacturing system, rather than relying solely on soft hydrogels to bear the entire mechanical load.

In terms of bone defect characteristics, extrusion-based bioprinting is more suitable for constructing bone repair constructs that require a larger volume and possess specific anatomical shapes or internal canal structures; it is particularly well-suited for scenarios requiring the simultaneous integration of dECM, high-viscosity hydrogels, and mineralized components. For large-volume defects with limited blood supply, multi-nozzle, coaxial, or embedded printing can be combined to construct vascular-related channels.^{46,47} However, in weight-bearing areas, extruding soft dECM hydrogels alone typically fails to provide sufficient early mechanical support; therefore, they are better suited for use in combination with polymer or inorganic scaffolds.

3.2 Inkjet Bioprinting

Inkjet bioprinting typically uses thermal, piezoelectric, or other on-demand jetting methods to deposit low-viscosity bio-ink in the form of discrete microdroplets at specified locations⁴⁸. This technology uses droplets as the basic printing unit and is characterized by relatively high printing speed, relatively low material consumption, and high precision in localized delivery; it can be used for the targeted loading of cells, proteins, or growth factors⁴⁹. Consequently, inkjet bioprinting is particularly well-suited for creating compositional gradients, locally loading bioactive substances, or achieving the spatial distribution of various cell types within a construct.

In bone tissue engineering, inkjet printing is better suited to play the role of “precise delivery.” For example, osteogenesis-related cells, angiogenesis-related cells, or bioactive factors can be deposited in specific regions of a construct, thereby spatially forming functional zones with distinct regenerative tasks⁵⁰. Animal studies have shown that the spatial patterning of osteogenesis-related factors via inkjet printing can influence the location and extent of local new bone formation in cranial defect models⁵¹. Therefore, for the bone-cartilage interface, the edges of bone defects, or areas requiring localized promotion of vascularization, inkjet technology offers certain advantages in achieving localized regulation of bioactivity.

However, inkjet bioprinting is more sensitive to the rheological window of bio-inks, typically requiring materials to maintain low viscosity and good flowability; when viscosity is too high, problems such as nozzle clogging, unstable droplet formation, or limited cell loading are likely to occur⁵². This characteristic limit its direct application to high-concentration dECM, inorganic

particle composites, and high-viscosity polymer systems. In particular, when mineralized particles are added, further coordination is often required among material viscosity, particle dispersion, and droplet formation behavior⁵³. At the same time, constructs formed solely by droplet deposition generally exhibit weaker mechanical properties^{2,54}. Therefore, in bone repair, inkjet technology is more suitable as an auxiliary manufacturing method, used in combination with other support structures or printing methods, rather than being used alone for constructing large-scale, load-bearing bone defects.

Therefore, from the perspective of clinical defect characteristics, inkjet printing is more suitable for bone defects where the primary load-bearing structure is already provided by a scaffold or host tissue, and where high-precision delivery of cells or bioactive factors to specific areas is required. For example, at the margins of craniofacial bone defects, in osteochondral transition zones, or in areas with insufficient local vascularization, inkjet printing's droplet deposition can be utilized to achieve spatial functional regulation. Conversely, for bone substitute structures that require large volumes, high viscosity, or the direct bearing of mechanical loads, inkjet printing is generally not suitable as the sole manufacturing method for the main structure⁵⁵.

3.3 Photopolymerization Bioprinting

Photopolymerization bioprinting primarily includes technologies such as stereolithography and digital light processing (DLP)⁵⁶. The basic principle involves using light of a specific wavelength to induce cross-linking in photosensitive biomaterials and performing selective curing layer by layer or region by region based on a digital model, thereby forming three-dimensional structures with predetermined geometries. Compared to extrusion-based printing, photopolymerization technologies typically offer higher fabrication precision and are particularly well-suited for constructing complex pore structures, microchannels, and fine interface structures⁵⁷.

In the field of bone repair, the advantage of photopolymerization printing lies in its ability to precisely control pore size, internal channels, and local structural variations, which holds potential value for simulating trabecular bone arrangement, medullary cavity-like spaces, or bone-cartilage interfaces. For dECM systems, it is typically necessary to introduce photo-crosslinkable groups or blend the material with photosensitive hydrogels to enable rapid formation of a stable network

upon light exposure⁵⁸. Such treatments can address the issues of post-printing collapse and insufficient structural stability in natural dECM while preserving the tissue-related biological information of the dECM⁵⁹; simultaneously, light-mediated crosslinking provides further opportunities to optimize the mechanical performance and shape retention of dECM constructs⁶⁰.

However, photopolymerization technology is not without its limitations. First, the photochemical modification and crosslinking processes may alter the properties of some natural matrix components to a certain extent; therefore, it is necessary to balance the biological integrity and structural stability of the material after modification^{60,61}. Second, the concentration of the photoinitiator, exposure time, light intensity, and crosslinking density may all affect cell viability and the material's degradation behavior⁶². Insufficient crosslinking leads to inadequate stability of the construct, while excessive crosslinking may restrict cell migration and matrix remodeling. Therefore, the design of photopolymerizable dECM bio-inks focuses not merely on increasing curing speed, but on ensuring a reasonable balance among printing precision, cell compatibility, and subsequent tissue remodeling^{61,63}.

In terms of defect morphology and structural requirements, photopolymerization technology is better suited for bone repair scenarios involving complex geometric structures and high spatial precision, such as constructs requiring precise control over pores, microchannels, trabecular-like structures, or the bone-cartilage interface. Its advantages are even more pronounced when the restoration goal relies heavily on spatial control of microscale structures or vascular-related channels. However, for large-volume, load-bearing bone defects, light-cured dECM hydrogels remain limited by material systems, scale-up challenges, and mechanical properties, and typically require combination with other reinforcing materials or manufacturing strategies⁶⁴.

3.4 Laser-Assisted Bioprinting

Laser-assisted bioprinting is typically based on principles such as laser-induced forward transfer (LIFT). By directing a pulsed laser at energy-absorbing regions of the donor layer, it generates localized transient pressure that propels biomaterials from the donor layer to the recipient substrate, thereby enabling nozzle-free deposition of cells and biomaterials⁶⁵. Unlike methods that rely on continuous extrusion through a nozzle, this technology enables the targeted transfer of

materials via microdroplets or microjets, thereby reducing, to some extent, the risk of clogging within the nozzle caused by high-viscosity materials, cell aggregates, or particulate components⁶⁵. Since the printing process does not involve the continuous flow and compression typical of traditional nozzles, laser-assisted printing can also mitigate some of the mechanical shear exposure caused by the extrusion process⁶⁶.

Laser-assisted printing offers advantages in terms of cell positioning accuracy and control over local cell distribution, making it suitable for constructing spatially heterogeneous tissue structures such as complex cell arrangements, locally vascularized regions, or bone-cartilage transition interfaces⁶⁷. In bone repair-related research, *in vivo* experiments have utilized laser-assisted methods to directly deposit mesenchymal stromal cells into bone defect sites, and a promotional effect on local bone regeneration has been observed⁶⁸. However, this technology imposes stringent requirements on laser parameters, donor layer structure, and recipient substrate conditions. Equipment costs and operational complexity are relatively high, and large-scale construction efficiency and standardized manufacturing capabilities remain limited⁶⁹. Consequently, its current application in bone tissue engineering is primarily focused on high-precision cell patterning, the construction of localized tissue interfaces, and mechanism validation, and it still falls short of routine clinical manufacturing⁶⁵. For dECM bio-inks, laser-assisted technology holds some exploratory value but is not yet the most commonly used manufacturing route for bone repair.

Therefore, laser-assisted bioprinting is more suitable for repairing bone defects that are relatively localized but require precise cellular spatial positioning or pre-vascularized tissue design, rather than for the direct fabrication of large-volume load-bearing scaffolds. For example, mesenchymal stem cells or endothelial cells can be precisely arranged in specific areas of a bone defect to form a predetermined cell distribution and microvascular network. Its application value is particularly prominent for localized bone defects with insufficient vascularization, tissue interfaces, or areas requiring precise cell patterning.

3.5 Hybrid and Multi-Material Bioprinting

A practical challenge in bone tissue engineering is that a single material often cannot simultaneously meet multiple requirements, such as mechanical support, cell compatibility, mineralization induction, and vascularization⁷⁰. Given this limitation, hybrid and multi-material

bioprinting has gradually emerged as an important direction in the design of bone repair constructs⁷¹. This strategy typically involves constructing a support framework from polymers or ceramic materials with high mechanical strength, while incorporating cell-loaded hydrogels, dECM bio-inks, and necessary bioactive components into its interior or on its surface, thereby forming a composite system in which “a rigid scaffold provides structural stability and a soft matrix provides the biological microenvironment”⁷².

For large-volume bone defects, this design generally better meets practical repair needs⁷³. An external scaffold formed from biodegradable polymers or inorganic materials can bear part of the mechanical load in the early stages⁷⁰, while a dECM-based hydrogel provides a relatively favorable microenvironment for cell adhesion, migration, and subsequent tissue remodeling⁷⁴. By further integrating techniques such as multi-nozzle printing, coaxial printing, or embedded printing, it is also possible to construct complex structures with compositional gradients, vascular channels, or layered bone-cartilage structures to meet the regenerative needs of different regions^{36,75}.

However, multi-material printing does not mean that more material types are necessarily better⁷⁶. Interactions at material interfaces, differences in degradation rates, compatibility of printing parameters, and *in vivo* reactions following implantation can all affect the final outcome of the construct⁷⁷. Particularly in bone repair, if a rigid scaffold occupies the regeneration space for an extended period or fails to integrate effectively with the newly formed bone, it may actually interfere with local tissue replacement and subsequent bone remodeling⁷⁸. Therefore, the key to hybrid printing lies not merely in “combining materials,” but in establishing a rational structure-material-function relationship based on the stress conditions of the bone defect, the stage of regeneration, and the requirements of tissue interfaces.

From a clinical translation perspective, hybrid and multi-material bioprinting is more suitable for large-volume, structurally complex, or weight-bearing bone defects, particularly in cases where early mechanical stability, the osteogenic microenvironment, and vascularization must be addressed simultaneously. By spatially combining a scaffold formed from high-strength polymers or mineralized materials with cell-loaded dECM hydrogels, vascularized regions, or bioactive components, these elements can respectively provide structural support and regulate regeneration.⁷⁹ Therefore, for scenarios such as segmental long bone defects—where the

requirements cannot be met by a single soft bio-ink—hybrid bioprinting offers greater translational potential.

To facilitate a comparison of the characteristics of different bioprinting technologies, Table 1 summarizes the main advantages, limitations, applicability in bone repair, and key influencing factors of the five categories of technologies mentioned above (Table 1). Overall, extrusion, inkjet, photopolymerization, laser-assisted, and hybrid printing technologies each have their own applicable scope; the choice of technology should be guided by the specific bone repair task rather than a mere preference for a particular manufacturing method^{36,80}. For dECM bioprinting, the most critical issue is not the selection of a specific technology, but rather the rational determination of material composition, cell loading methods, cross-linking mechanisms, and spatial structure based on bone repair requirements⁸¹. Subsequent chapters will further discuss the characteristics of dECM from different sources, as well as how to improve its printability and enhance bone regeneration capabilities through processing methods, chemical modification, loading of bioactive substances, and biomimetic structural design⁸².

However, bioprinting methods and dECM sources are not two independent factors; rather, they jointly determine the processing window of the bio-ink and the properties of the final construct. dECMs from different tissue sources vary in terms of collagen composition, glycosaminoglycan content, degree of mineralization, and microstructure. These tissue-specific characteristics further influence the material's viscosity, rheological behavior, gelation mechanism, cross-linking requirements, and structural retention capacity after printing.⁸³ Therefore, a single printing technology may not necessarily be suitable for dECMs from all sources. For example, bone-derived dECMs, which contain a higher proportion of mineralized or particulate components, typically require greater attention to rheological stability and nozzle compatibility during the extrusion process, whereas hydrogel systems formed from cartilage- and soft tissue-derived dECMs require a greater focus on addressing crosslinking efficiency and shape retention issues; When constructing osteochondral or bone-soft tissue interfaces, dECMs from different sources can be further combined with multi-material or segmented printing. Consequently, the selection of printing technology should be considered in conjunction with the dECM's tissue origin, the processed material state, and the target tissue for repair, rather than being determined in isolation⁸⁴.

Table 1. Comparison of major bioprinting techniques for dECM-based bone repair.

Printing technique	Main advantages	Main limitations	Applications in bone repair	Key parameters/factors	Reference
Extrusion-based bioprinting	Broad viscosity range; suitable for high-concentration and composite bioinks; enables fabrication of porous scaffolds	Shear stress may affect cell viability; limited mechanical support of soft hydrogels	Highly compatible with dECM and readily combined with ceramics or polymers	Nozzle diameter, bioink viscosity, printing pressure	[10, 42, 45, 47, 48]
Inkjet-based bioprinting	High printing speed; low material consumption; high precision for localized delivery	Limited by bioink viscosity; risk of nozzle clogging or unstable droplet formation; relatively weak mechanical properties	Suitable for generating compositional gradients and spatial delivery of cells or bioactive factors	Bioink viscosity, particle dispersion, droplet formation behavior	[2, 50-56]
Light-based bioprinting	High printing resolution; suitable for complex pores, microchannels, and precise interfaces	Photochemical modification and crosslinking conditions may affect matrix properties and cell viability	Suitable for constructing trabecular-like structures, bone marrow cavity-like spaces, and osteochondral interfaces	Photoinitiator concentration, exposure time, light intensity, crosslinking density	[58, 59, 61-65]
Laser-assisted bioprinting	Nozzle-free; reduced risk of clogging; high cell-positioning accuracy	High equipment cost and operational complexity; limited scalability and standardization	Suitable for high-precision cell patterning and localized tissue-interface construction	Laser parameters, donor-layer structure, receiving-substrate conditions	[67-70]
Hybrid/multi-material bioprinting	Integrates structural stability with a favorable biological microenvironment; enables gradient, channel, and layered structures	Challenges in material interfaces, degradation-rate matching, and printing-parameter compatibility	Suitable for large-volume bone defects and complex composite constructs	Material interfacial bonding, degradation rate, printing-parameter compatibility	[37, 71, 74-80]

4. Characteristics of Decellularized Extracellular Matrices from Different Sources and Their Application Value in Bone Repair

The value of dECM lies not only in its origin from natural tissues, but also in the fact that the matrix composition, microstructure, and biological information retained by different tissues vary⁸⁵. In bone repair, the source of the material directly influences the mineralization potential of the construct, its cell adhesion properties, its compatibility with tissue interfaces, and the subsequent remodeling process⁸⁶. Therefore, the selection of dECM should not be based solely on “whether it possesses natural biological activity,” but should be determined by considering the defect site, loading conditions, tissue interfaces, and the intended repair goals.

4.1 Bone-Derived dECM

Bone-derived dECM is one of the most targeted materials for bone defect repair⁸⁷. The natural bone matrix consists primarily of type I collagen as its main organic component, while also containing various non-collagenous proteins, glycosaminoglycans, and mineralization-related matrix components¹. After decellularization, bone-derived dECM retains these tissue-specific characteristics to varying degrees, providing a microenvironment that closely resembles bone tissue for cell adhesion, migration, and osteogenic differentiation⁸⁸. Compared to general natural hydrogels, its advantage lies not merely in its “bone origin,” but in its ability to align the material’s composition more closely with the matrix requirements of the bone repair process.

It is important to note that bone-derived dECM is not a single, uniform material. Its final properties are influenced by multiple factors, including donor age, harvest site, retention of mineral phases, decellularization method, and enzymatic digestion conditions²³. Bone matrix that retains some mineral components typically provides better clues regarding mineralization; however, the material processing and printing processes are more prone to limitations such as particle dispersion, viscosity, and nozzle clogging^{11,86}; bone dECM that has been demineralized or thoroughly digested is more suitable for the preparation of hydrogels and bio-inks, but its early mechanical support capacity is often weak⁸⁹. Therefore, bone-derived dECM is better suited to serve as the “biologically functional phase” in bone repair constructs, working in conjunction with polymer scaffolds, bioceramics, or cross-linked systems to provide structural stability and regulate regeneration.

In clinical practice, bone-derived dECM is particularly suitable for bony defects where the osteogenic microenvironment is critical, such as segmental bone defects, cancellous bone defects, reconstruction following bone tumor resection, or filling and repair of bone fusion sites^{33,90}. However, for sites subject to high loads or with extensive defects, the value of the material cannot be determined solely based on its osteogenic potential; one must also evaluate its bonding mechanism with the scaffold material, its degradation rate, and its *in vivo* osseointegration performance⁹¹. In other words, the advantage of bone-derived dECM lies in providing the “right microenvironment” for new bone formation, rather than solely replacing all the mechanical functions required by mature bone.

4.2 Cartilage-Derived dECM

Cartilage-derived dECM is primarily characterized by components such as type II collagen, proteoglycans, and glycosaminoglycans. Its most significant value lies in maintaining the phenotype of cartilage-associated cells and mimicking the high-water-content, low-friction matrix microenvironment of cartilage tissue^{7,92}. For isolated bone defects, the primary goal of cartilage-derived dECM is not typically to enhance mineralization; however, its tissue specificity offers greater clinical value in the repair of articular surfaces, subchondral bone, or the osteochondral interface⁹³.

The challenge with osteochondral defects lies in the significant differences between the cartilage layer and the subchondral bone in terms of composition, mechanical properties, and repair requirements⁹⁴. While the use of bone-derived materials alone is beneficial for subchondral bone reconstruction, it fails to adequately mimic the matrix environment of the cartilage layer; conversely, the use of cartilage-derived dECM alone is insufficient to meet the mechanical and mineralization demands of the bony region⁸². Therefore, cartilage-derived dECM is more suitable for placement on the articular surface or in transitional zones, where it can be combined with bone-derived dECM, mineralizing components, or scaffolds to form layered or gradient structures^{95,96}.

In bioprinting systems, another function of cartilage-derived dECM is to provide cells with a relatively mild, cartilage-like microenvironment. When blended with hydrogels, it can be used to construct cell-loading layers, cartilage surface layers, or transition layers at the osteochondral interface⁹⁷. However, such materials generally suffer from limited strength after curing and insufficient long-term shape retention; therefore, they still require optimization through cross-

linking methods and support structures⁹⁸. The focus of their application should not be on “replacing bone materials,” but rather on improving continuity and functional integration between different tissue regions in osteochondral composite defects.

4.3 Soft Tissue and Cell-Derived dECM

In addition to bone and cartilage, soft tissues such as fat, skeletal muscle, tendons, and ligaments, as well as cells cultured in vitro, can all serve as sources of dECM^{85,99}. While these matrices typically lack the mineralization characteristics of bone tissue, they retain the tissue-specific collagen arrangements, cell adhesion sites, and tissue repair microenvironment¹⁰⁰. For bone repair, their significance lies more in improving cell adhesion, local tissue integration, and interface repair, rather than solely undertaking the task of mineralized bone regeneration.

For example, at tendon-bone attachment sites, in bone tunnels for ligament reconstruction, or in bone defects accompanied by extensive soft tissue injury, soft tissue-derived dECM can serve as an interface layer or supportive microenvironment material, helping to alleviate the lack of transition between hard tissue scaffolds and surrounding soft tissues¹⁰¹. dECM derived from adipose tissue and skeletal muscle can also be used to construct hydrogel systems with good cellular compatibility¹⁰; however, their contribution to osteogenesis typically still requires combination with bone-derived dECM, inorganic mineralizing components, or osteogenesis-related cells¹⁰².

Cell-derived dECM, on the other hand, exhibits a different set of characteristics: its composition can be regulated to a certain extent depending on cell type and in vitro culture conditions; it offers relatively good batch reproducibility; and it facilitates the creation of specific cellular microenvironments¹⁰³. However, the yield of cell-derived matrices is limited, and the degree of matrix maturation and compositional stability remain influenced by culture conditions⁸⁵. Existing research has shown that different cell combinations can form cell-derived ECM with varying matrix compositions, which in turn affects their ability to promote bone regeneration¹⁰⁴. At this stage, it is more suitable as a supplementary material or for mechanistic studies rather than as a primary material to directly replace that required for the repair of large-volume bone defects.

Unlike bone-derived dECM, which directly addresses bony defects, the value of cartilage-, soft tissue-, and cell-derived dECM in bone repair lies primarily in the reconstruction of specific

tissue interfaces and functional microenvironments. In recent years, tissue-specific dECMs have been used for bioprinting at gradient interfaces such as osteochondral and tendon-bone interfaces. By configuring cartilage-, tendon-, or bone-derived matrices in different spatial regions, these dECMs provide differentiated microenvironments that facilitate regional cell differentiation and tissue integration¹⁰⁵. Therefore, in complex bone defects, dECMs from different sources are better suited for spatial combination as complementary materials rather than simply serving as substitutes for bone-derived dECMs.

4.4 Selection Principles for dECM from Different Sources and Their Impact on Bioprinting

There is no absolute superiority or inferiority among dECMs from different sources; the key lies in whether they are well-suited to the specific bone defect repair task¹⁰⁶. In cases where the primary goal is bone defect repair or bone fusion, bone-derived dECM can provide a matrix environment that more closely resembles bone tissue; when the defect involves articular cartilage, subchondral bone, or the osteochondral interface, it is more appropriate to consider a zoned or gradient combination of cartilage-derived dECM and bone-derived materials⁹⁴. For complex defects accompanied by tendon, ligament, or surrounding soft tissue injuries, soft tissue-derived dECM can serve as an interface layer or auxiliary microenvironment material to improve the transition and integration between different tissues¹⁰⁷. The following are some representative studies on this topic^{27,39}.(Figure 3)

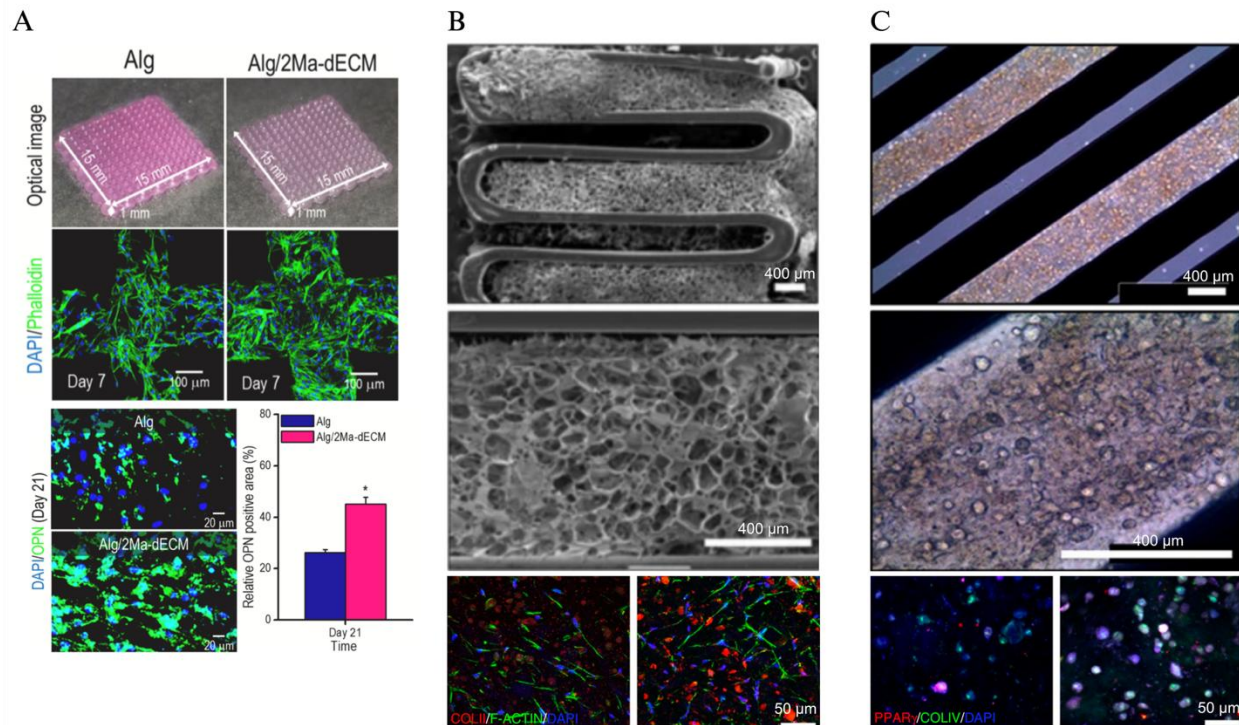


Figure 3. dECM from different sources for bone defect repair. (A) A typical application of osteogenic dECM bio-ink in the preparation of osteoblast-loaded constructs, demonstrating its suitability for reconstructing a bone-specific microenvironment. (B) A typical application of cartilage-derived dECM bio-ink, highlighting its value in maintaining cartilage-related properties and supporting osteochondral and interface repair. (C) A typical application of soft tissue-derived dECM bio-ink, demonstrating its role in tissue integration and microenvironmental support. Reprinted with permission from Jiun L et al and Falguni Pati et al.^{27, 39} Copyright ©2014 Springer Nature, 2020 Elsevier.

The choice of material should also be tailored to the biomechanical conditions at the defect site and the stage of repair. For weight-bearing defects, dECM typically needs to be combined with polymer scaffolds or inorganic materials to maintain early spatial stability and provide a foundation for subsequent cellular infiltration and vascularization¹⁰⁸; whereas for defects where tissue integration is the primary focus, greater emphasis should be placed on the material's pore connectivity, degradation behavior, and cellular compatibility. An ideal dECM construct should not function in the same way throughout the entire repair process; rather, it should maintain the basic structure in the early stages, support tissue formation in the middle stages, and gradually participate in the remodeling of new bone in the later stages¹⁰⁹.

In addition, the source of the dECM also influences the selection of bio-ink formulations and printing methods. Bone-derived dECM often requires addressing issues related to rheological stability and printability caused by mineralized components; cartilage- and soft tissue-derived dECM, on the other hand, are more prone to forming a hydrogel environment but typically require further improvement in shaping stability¹¹⁰. For multi-material printing, dECMs from different sources can also be configured in different regions of the construct to meet the differentiated requirements of bony regions, cartilaginous regions, or tissue interfaces¹¹¹.

Therefore, the selection of dECM sources should follow a sequential decision-making pathway of “defect type—tissue source—material processing—printing method—functional goal,” rather than being based solely on natural origin or a single osteogenic indicator. Bone-derived dECM places greater emphasis on the osteogenic microenvironment; cartilage-derived dECM is more suitable for the osteochondral interface; while soft tissue- and cell-derived dECM primarily address the needs for cell adhesion, tissue integration, and interface transition. Building on this foundation, further discussion is needed on how to transform dECM from different sources into functional bio-inks that combine printability, structural stability, and bone regeneration capacity through processing, material compositing, and structural design¹¹².

5. Advanced Strategies for Bioprinting with Decellularized Matrices

Once the compositional characteristics and scope of application of dECM from different sources have been clarified, the focus of research should shift from “which dECM to select” to “how to optimize dECM into a bioprinting system better suited for bone repair.” Natural dECM retains a certain amount of collagen networks, glycosaminoglycans, cell adhesion sites, and tissue-specific matrix components, providing a microenvironment that closely resembles the natural state for cell survival and new tissue formation¹⁰⁶. However, dECM hydrogels alone often suffer from issues such as insufficient viscosity, poor shape retention after printing, limited mechanical properties, and difficult-to-control degradation behavior^{11,113}, making it difficult for them to independently meet the requirements of bone defect repair for early spatial support, mineralization, and long-term tissue integration.

Therefore, advanced strategies for dECM bioprinting should not simply focus on increasing material complexity, but rather on the practical needs of bone repair through the synergistic design of material types, decellularization processes, chemical cross-linking, bioactive substance loading,

and spatial structures¹¹⁴. An ideal construct should exhibit stable rheological properties and formability during the printing process, maintain the defect space and basic mechanical stability in the early post-implantation period, and—in subsequent stages—support cellular infiltration, vascular ingrowth, matrix deposition, and new bone remodeling through the regulation of active components, including extracellular vesicles¹⁰⁷, ultimately achieving good integration with the host tissue.

From the perspective of the temporal progression of bone healing, the aforementioned advanced strategies do not act on independent stages separately, but rather undertake distinct yet interconnected tasks across the three phases of inflammation, repair, and remodeling. The design focus during the inflammatory phase lies in controlling material-related immune stimulation, maintaining early spatial stability, and creating a suitable environment for subsequent cellular infiltration; therefore, it primarily involves thorough yet gentle decellularization, moderate cross-linking, and the use of biocompatible material composites. Upon entering the repair phase, cell recruitment, angiogenesis, and new bone formation become the primary requirements. At this stage, the roles of dECM composites with mineralizing materials, bioactive factor or cell loading, and the design of vascular channels and gradient structures become more prominent. In the remodeling phase, further attention must be paid to the dynamic balance between material degradation, mechanical support, and new bone replacement; therefore, the degree of cross-linking, the degradation behavior of composite scaffolds, and the long-term evolution of pore and interface structures are particularly important. These strategies typically span multiple healing stages, and their significance lies in enabling the functional evolution of the construct—through spatiotemporal coordination of materials and structures—to gradually shift from “early stabilization” to “tissue formation” and ultimately transition to “host bone remodeling and integration” as the bone repair process progresses¹¹⁵.

5.1 Material Types and Composite Optimization Strategies for dECM Bio-Inks

Single-component soluble dECM bio-inks represent a relatively basic form of material. Typically, decellularized tissue is prepared into a pre-gel solution through pulverization, enzymatic digestion, and pH neutralization, and then cured through temperature-induced self-assembly, changes in the ionic environment, or auxiliary cross-linking^{6,39,116}. This type of material retains

some components of the natural matrix and exhibits good cellular compatibility, making it suitable for cell loading, localized filling, or as a functional component within composite constructs. Its limitations include a relatively narrow printing window, limited gelation rates and shape retention capabilities, and difficulty in precisely controlling mechanical properties and degradation behavior^{10,11,117}. Therefore, single-component dECM is more suitable for non-load-bearing areas, cell delivery layers, or the bioactive portions of composite scaffolds, but is not recommended as the primary support material for load-bearing bone defects on its own.

Specifically, these dECM constructs, which have relatively limited mechanical properties, are more suitable for low-load bone defects—such as those in the craniofacial region—as well as areas primarily responsible for local tissue regeneration and interface integration, such as the osteochondral interface and the tendon-bone interface. For weight-bearing sites, such as segmental defects in long bones, they are better suited as bioactive filling layers when used in combination with high-strength polymers or inorganic scaffolds, rather than being relied upon to bear the primary mechanical load on their own¹¹⁸.

From the perspective of the healing stages, the material composite strategy spans the entire bone repair process: in the early stage, its primary role is to maintain the defect space and ensure necessary mechanical stability; in the middle stage, it supports cell adhesion, vascularization, and new bone formation through dECM and mineralized components; and in the late stage, it must gradually transfer load-bearing and structural functions to the newly formed bone tissue through an appropriate degradation rate¹¹⁹.

Combining dECM with natural polymeric materials is a common strategy for improving the printing performance of bio-inks. Materials such as gelatin, methacrylated gelatin, alginate, chitosan, and hyaluronic acid can improve the continuity of dECM ink during extrusion and the interlayer stability after printing by increasing the viscosity of the system, adding cross-linking sites, or shortening the gelation time¹⁵. Among these, gelatin or methacrylated gelatin can improve rheological properties during low-temperature printing and, through photocrosslinking after deposition, further enhance the morphological stability of the printed structures¹²⁰; alginate, on the other hand, enables rapid curing through ionic crosslinking. Previous studies have shown that combining dECM with GelMA, hyaluronic acid derivatives, and other materials can improve formability while maintaining good cell compatibility^{121,122}. However, the proportion of polymeric

materials should not be too high, as this may reduce the relative contribution of dECM in the construct and affect material degradation and subsequent tissue remodeling.

When combined with synthetic polymer materials, dECM is better suited to address the issue of early mechanical support in bone repair. Materials such as polycaprolactone, polylactic acid, and polylactic-co-glycolic acid copolymers possess relatively stable mechanical properties and good processability, and can be 3D-printed into external frameworks, porous lattices, or primary load-bearing scaffolds¹⁰⁹; dECM hydrogels, on the other hand, are filled within the pores or applied to the surface of the scaffold, primarily serving to enhance cell adhesion, nutrient exchange, and the local regenerative microenvironment³². This “rigid scaffold–soft matrix” model achieves a relative division of labor between mechanical performance and bioactivity: the polymer framework maintains the shape of the construct and bears part of the load, while the dECM provides cells and newly formed tissue with a microenvironment that more closely resembles the natural matrix³³. The use of thermoplastic materials such as PCL to construct protective layers or support frameworks has been shown to enhance the structural stability of cell-loaded constructs¹²³; meanwhile, the combined use of PCL and decellularized bone matrix has also demonstrated potential for balancing structural design with the bone regenerative microenvironment^{108,124}. Therefore, for large bone defects, non-union fractures, or situations requiring long-term maintenance of the defect space, such composite systems generally offer greater clinical value.

Combining dECM with inorganic mineralization materials represents an optimized approach that better aligns with the characteristics of bone tissue. Hydroxyapatite, β -tricalcium phosphate, bioactive glass, and other calcium-phosphate-based materials can enhance the mineralization properties, bone conduction capacity, and compressive modulus of the construct, while providing an interface environment for extracellular matrix deposition that more closely resembles that of bone tissue¹⁴. Composites of dECM and inorganic components can balance the stability of the construct with cellular compatibility, mitigating the insufficient biological adaptability associated with high inorganic content⁴²; simultaneously, composites of bone-derived dECM with polymers, ceramics, or other bioactive components also help balance the material’s mechanical performance and regenerative function³⁵. Previous studies have shown that composite scaffolds composed of β -tricalcium phosphate, decellularized bone matrix, and PCL can form a bone repair system that synergistically combines physical support and bioactivity¹²⁵; integrating extracellular matrix with

amorphous magnesium phosphate into a bio-ink can also be used for craniofacial bone tissue engineering¹²⁶. For weight-bearing or large-volume bone defects, the synergistic combination of dECM, calcium-phosphate materials, and stable polymer frameworks is highly feasible; however, particle size and addition ratios must still be controlled to prevent nozzle clogging, filament breakage, or shear damage to cells.

Granular or microfibrillar dECM represents another promising form of material. Incorporating dECM—prepared as microparticles, microfibers, or fine fragments—into a base hydrogel can enhance the material's viscoelasticity and shear-thinning properties, thereby improving flow behavior and deposition stability during the printing process¹¹⁷. At the same time, particulate dECM increases the local contact area between cells and the native matrix, providing more microenvironmental sites for cell adhesion, migration, and matrix deposition, while offering greater flexibility for the design of subsequent composite materials¹⁵. As a representative example of a dECM composite bio-ink derived from bone particles, the combination of decellularized bone particles and gelatin can form a three-dimensional scaffold with certain printability, and this combination influences the scaffold's printing parameters and microstructural characteristics^{127,128}(Figure 4); adding extracellular matrix microparticles to a GelMA system can also improve printing resolution and self-supporting capability under room-temperature conditions¹²⁹. This strategy is suitable for filling irregular bone defects, minimally invasive injection, and extrusion-based printing; however, particle size distribution and loading levels must still be controlled to avoid compromising nozzle patency, printing accuracy, and the internal homogeneity of the printed structures.

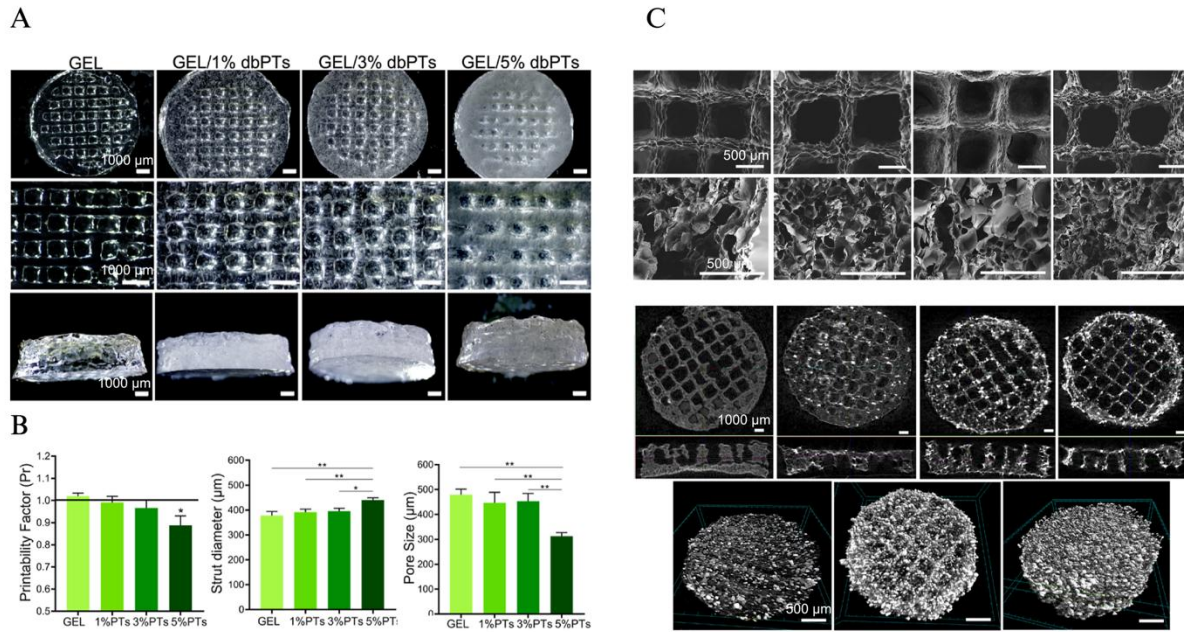


Figure 4. Representative particulate bone dECM/gelatin composite scaffold system. (A) Morphology of printed gelatin scaffolds containing different concentrations of decellularized bone particles. (B) Printability factor, strut diameter, and pore size of the printed constructs. (C) Scanning electron microscopy and micro-computed tomography characterization of scaffold porosity, surface morphology, and particle distribution. Reprinted with permission from A. Kara et al.¹²⁸ Copyright ©2022, Elsevier.

In addition, multicomponent and gradient-type dECM constructs can be further adapted to meet the demands of complex bone repair. By adjusting the degree of mineralization, the proportion of inorganic materials, the cross-linking strength, and the porosity in different regions¹⁴, it is possible to create peripheral support zones, internal regeneration zones, and vascular channel zones within a single construct, thereby achieving a spatial division of labor between mechanical stability and the regenerative microenvironment²⁶. For example, the peripheral region can feature a higher inorganic content and a relatively dense structure to maintain early stability; the internal region, on the other hand, can be rich in dECM and feature more interconnected pores to facilitate cell infiltration, mass exchange, and vascular ingrowth³⁴. Multi-material dECM bioprinting has demonstrated the feasibility of regulating cell behavior and tissue formation through the spatial distribution of materials¹³⁰; meanwhile, the integration of cells, endothelial cell spheroids, and dECM/ β -tricalcium phosphate composite struts into a single construct also provides a reference for vascularized bone tissue engineering¹³¹. Such designs emphasize the synergistic optimization

of material form and structural configuration, which can be clearly linked to the selection of dECM sources discussed in Section 4.

5.2 dECM Processing Methods: Synergistic Optimization of Physical, Chemical, and Enzymatic Approaches

High-quality dECM is the foundation for subsequent printing, modification, and functionalization. The core of the decellularization process is to remove cells, nucleic acids, and potentially immunogenic components as thoroughly as possible while maximizing the retention of collagen fibers, glycosaminoglycans, adhesion molecules, and tissue-specific microstructures^{6,10}. Insufficient processing can result in residual cell debris and nucleic acids, increasing biosafety risks^{20,132}; conversely, excessive processing may lead to collagen denaturation, glycosaminoglycan loss, and reduced gel-forming capacity. Therefore, decellularization is not necessarily better the more thorough it is; rather, a balance must be struck between the removal of cellular components and the preservation of matrix function¹³³.

Physical methods primarily include repeated freeze-thaw cycles, osmotic gradients, mechanical agitation, sonication, pressure treatment, and tissue homogenization^{11,113}. Repeated freeze-thaw cycles disrupt cell membranes through ice crystal formation, while osmotic gradients can induce cell swelling and rupture; mechanical homogenization or grinding increases tissue surface area and improves penetration of subsequent treatment solutions^{116,134}. For dense tissues such as bone and cartilage, mincing, grinding, or cryopulverization are typically essential prerequisites for chemical decellularization and enzymatic digestion. Physical methods leave fewer chemical residues and have a relatively limited direct impact on matrix components; however, when used alone, they often fail to thoroughly remove deep-seated cells and nuclear components, and are therefore frequently employed as preliminary steps in combined treatment protocols¹³⁵.

Chemical methods primarily utilize detergents, salt solutions, acid-base solutions, or organic solvents to disrupt cell membranes and dissolve intracellular components^{14,27}. Ionic detergents have strong decellularization capabilities but may damage collagen fibers, reduce glycosaminoglycans, and increase the difficulty of rinsing and residue control; nonionic detergents are milder and better preserve the matrix structure, but their efficiency may be lower³³. For lipid-

rich tissues, degreasing is also critical to minimize the impact of lipid residues on material uniformity, rheology, and gelation. For bone-derived dECM, a balance must be struck between demineralization and mineral retention: the former facilitates tissue softening, pulverization, and uniform enzymatic digestion, while the latter more closely mimics the bone tissue environment but increases the difficulty of processing and printing. Therefore, the treatment protocol should be determined based on the mechanical requirements of the construct and whether it is composited with inorganic materials¹³⁶.

Enzymatic methods are commonly used to remove residual nucleic acids, cellular proteins, and some lipid components^{11,13}. Nucleases reduce residual DNA and RNA and are a critical step in ensuring the biosafety of the material; proteases aid in the separation of cells from the matrix, but prolonged treatment can damage collagen and other functional components; lipases are more suitable for tissues with high lipid content^{15,117}. For dECM intended for bio-ink preparation, the extent of enzymatic treatment also affects molecular chain length, viscosity, and heat-induced gelling ability. Excessive enzymatic hydrolysis can make the material too thin, making it difficult to maintain the print filament; insufficient enzymatic hydrolysis may result in solution heterogeneity, particulate residue, and nozzle clogging¹³⁷.

Therefore, the preparation of dECM more suitable for bioprinting typically involves a combination of physical, chemical, and enzymatic methods: first, physical treatment is used to enhance tissue permeability; next, relatively mild chemical methods are employed to remove major cellular components; then, nucleases are used to further reduce residual nucleic acids; and finally, the material is thoroughly rinsed, freeze-dried, pulverized, enzymatically digested, and neutralized to prepare the bio-ink^{12,32}. In addition to assessing the degree of decellularization histologically, a comprehensive evaluation should include residual DNA, retained collagen and glycosaminoglycans, solution viscosity, gelation time, printing resolution, crosslinking stability, cell compatibility, and degradation behavior^{30,31}. Only by simultaneously incorporating decellularization quality and printing performance into the evaluation can one avoid obtaining materials that are “sufficiently decellularized but unsuitable for printing”¹³⁸.

In summary, from the perspective of bone healing stages, the quality of dECM treatment primarily determines immune compatibility and inflammatory responses during the early post-implantation period. At the same time, by preserving collagen, glycosaminoglycans, and tissue-

specific matrix components, it lays the foundation for cell adhesion, migration, and tissue formation during the subsequent repair phase; its effects actually begin during the inflammatory phase and extend into the subsequent regenerative stages⁴⁷.

5.3 Chemical Modification Strategies for dECM

Natural dECM exhibits good biological activity, but its cross-linking capacity and structural stability are typically insufficient^{39,116}. The goal of chemical modification is not to completely artificialize the dECM, but rather to enhance the material's printability, forming stability, controllable degradation, interfacial adhesion, and ability to bind active substances while preserving its primary biological advantages^{135,136}. For bone repair constructs, ideal modification should allow the material to set relatively quickly after printing, maintain its basic structure during the early stages of implantation, and undergo gradual degradation and remodeling as new bone forms¹³⁹.

Methacrylation is a common modification method. By introducing photo-crosslinkable groups into the dECM molecular chains, the material can form a relatively stable cross-linked network upon exposure to light after printing, thereby improving interlayer bonding and overall shape retention^{120,140}. This method is suitable for secondary curing after extrusion printing and can also be used in photopolymerization bioprinting systems¹⁴¹. For bone repair, methacrylation can reduce the risk of collapse in flexible dECM constructs during culture or in the early post-implantation period, and improve the spatial anchoring of cells and inorganic particles within the construct. However, the degree of modification should not be excessive, as this may result in pore shrinkage, excessively slow degradation, and restricted cell migration. In a representative study on the methacrylation of bone-derived dECM, Parthiban et al. subjected demineralized and decellularized human bone matrix to methacrylation to prepare BoneMA hydrogels capable of crosslinking under visible light; their mechanical properties can be adjusted by the duration of light exposure, and they support DLP printing of cell-loaded structures while maintaining high cell viability¹⁴². (Figure 5)

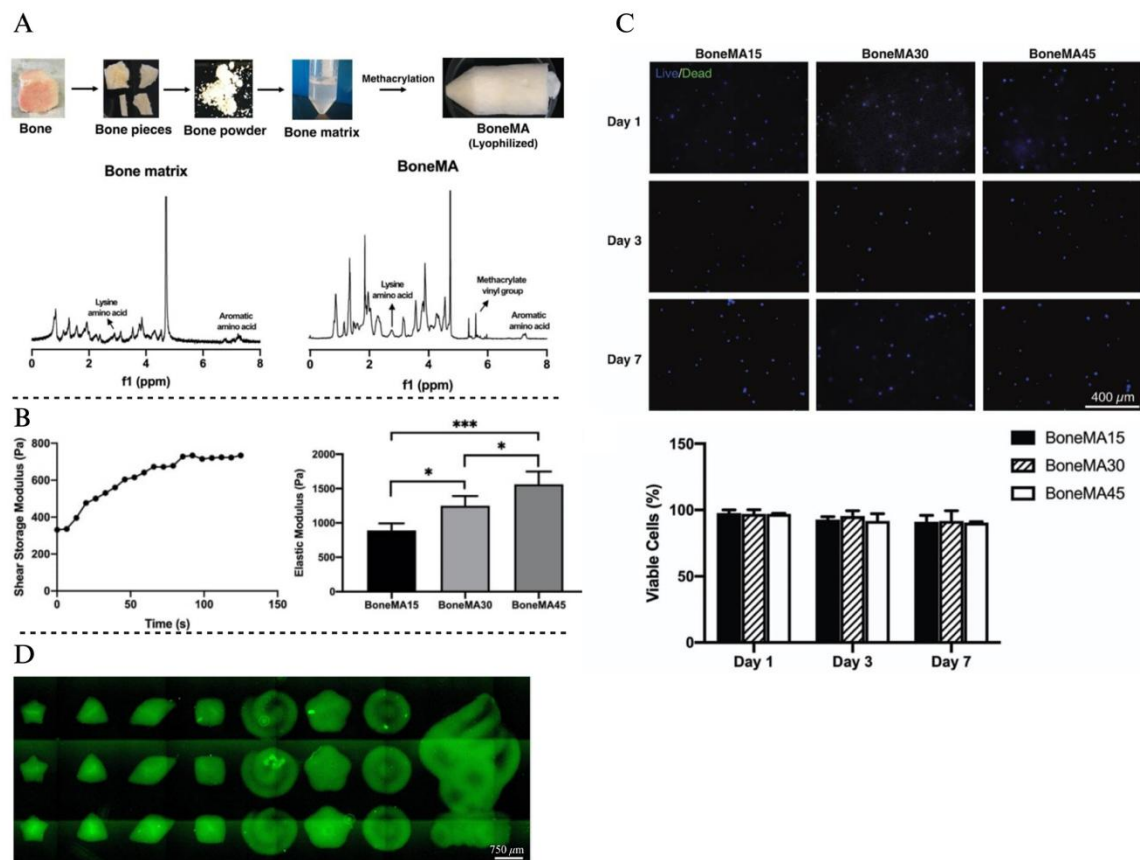


Figure 5. Representative methacrylation and light-mediated crosslinking of bone-derived dECM for bioprinting. (A) Schematic preparation of methacrylated bone-derived extracellular matrix hydrogel (BoneMA) from demineralized and decellularized human bone matrix, together with proton nuclear magnetic resonance spectra confirming the introduction of methacrylate groups. (B) Real-time photorheological analysis and elastic modulus measurements demonstrating light-mediated gelation and exposure time-dependent mechanical tunability of BoneMA. (C) Representative live/dead images and quantitative cell viability analysis of human dental pulp stem cells encapsulated in BoneMA hydrogels crosslinked for different durations. (D) Representative fluorescence images of cell-laden BoneMA constructs with different geometries fabricated using digital light processing bioprinting. Reprinted with permission from Parthiban SP et al.¹⁴² Copyright ©2021, Institute of Physics Publishing.

Dynamic covalent cross-linking strategies can endow materials with certain adaptive and self-healing capabilities⁴⁰. For example, introducing aldehyde groups after moderate oxidation, followed by reaction with amino-containing components, can form a cross-linked network with dynamic properties¹⁴³. Such materials can recover their structure to a certain extent after being subjected to shear forces, making them suitable for filling irregular bone defects, in-situ gelation, or bonding to complex surfaces^{127,137}. For bone repair, dynamic crosslinking helps improve the material's tolerance to local deformation and minor displacements; however, the degree of

oxidation must be strictly controlled to avoid damaging collagen structures and natural adhesion sites.

Crosslinking methods such as carbodiimide crosslinking and quinidine crosslinking can enhance the hydrolytic stability and mechanical stability of the material network¹⁴⁴, making them suitable for bone regeneration constructs that require long-term shape retention. Compared to purely physical gels, chemical cross-linking can reduce early swelling and excessive degradation⁶⁰; however, excessive cross-linking may render the construct too dense, hindering cell infiltration and vascularization¹³⁴. Therefore, the degree of cross-linking should be tailored to the defect size, construct thickness, material degradation timeline, and expected repair duration, rather than simply pursuing higher mechanical properties.

In addition, the introduction of catechol or dopamine-like groups, or the construction of composite networks with adhesive properties, can enhance the binding of dECM to moist tissue surfaces, inorganic particles, and polymeric scaffolds, thereby improving the interfacial stability between the construct and the host bone or support framework¹⁴⁵. Heparinization, sulfation, or the introduction of charged groups can improve the material's binding efficiency for protein-based bioactive factors and prolong their local retention time¹⁴⁶. In bone repair constructs, such interface adhesion and sustained-release designs can also be synergistically configured with mineralized materials, cell loading, or pre-vascularized regions^{130,131}. Overall, chemical modification should adhere to the principles of moderation, controllability, and evaluability, enhancing both the material's processability and structural stability while avoiding residual reagents, excessive cross-linking, or excessive masking of the native matrix's functions.

Chemical modification primarily plays a role in regulating structure across different stages: moderate cross-linking helps maintain the integrity of the construct during the early implantation phase, while its cross-linking density and degradation characteristics further influence cell migration and vascular ingrowth during the repair phase, as well as the gradual replacement of the material by host tissue during the remodeling phase; therefore, optimizing the material solely to improve its initial mechanical properties should be avoided²¹.

5.4 Synergistic Enhancement Strategies Using Bioactive Substances

Bone repair is a continuous process comprising an early inflammatory response, cell migration, angiogenesis, matrix deposition, mineralization, and long-term remodeling^{106,113}. dECM provides the foundational microenvironment; however, in cases of large-volume bone defects, infectious bone defects, poor local blood supply, or poor bone quality, relying solely on the material itself is often insufficient to meet the demands of multistage repair^{108,131}. Integrating growth factors, cells, exosomes, or drugs into dECM constructs can transform them from passive scaffolds into regenerative platforms capable of local regulation^{34,147}.

Growth factor loading is primarily used to enhance local osteogenesis, vascularization, or soft tissue integration. Osteogenic factors promote the formation of osteoid tissue, while vascular-related factors help improve the early nutrient supply to large-volume constructs¹⁰⁶. Components in the dECM, such as collagen and glycosaminoglycans, possess a certain capacity for protein binding, which can reduce the risk of sudden release caused by the rapid diffusion of active factors^{146,148}. Combining these approaches with methods such as microsphere loading, adsorption onto mineralized particles, layered printing, or chemical immobilization can achieve relatively slow and localized release³. It is important to note that higher doses of growth factors are not necessarily better; excessive amounts or uncontrolled release may lead to abnormal bone formation, local inflammation, or adverse reactions in surrounding tissues. Therefore, a more reasonable strategy involves low-dose, localized, and controlled release rather than simply increasing the loading amount.

Cell loading is one of the key advantages of dECM bioprinting. Bone marrow-derived mesenchymal stem cells, adipose-derived stromal cells, osteogenesis-related cells, and angiogenesis-related cells are all potential candidate cells^{39,116,127}. The natural adhesion sites and flexible microenvironment provided by dECM help mitigate shear stress during the printing process and support early cell survival and matrix secretion³⁹. For large-volume bone constructs, osteogenesis-related cells and vascular-related cells can be spatially partitioned or arranged in a synergistic manner, allowing the regenerative regions within the construct and the areas surrounding vascular channels to perform different functions¹³¹. For osteochondral repair, specific cell types can be allocated to different regions based on the tissue requirements at various levels¹³. It is important to note that cell-loaded constructs remain subject to limitations such as cell source, expansion efficiency, batch consistency, storage and transportation, and regulatory requirements;

therefore, their practical application requires balancing biological benefits against preparation complexity.

Exosomes offer an alternative approach to overcoming the challenges associated with live-cell applications. Exosomes can carry various bioactive components, including proteins, lipids, and nucleic acids, and are involved in regulating local cellular behavior and the tissue microenvironment¹⁰⁷. Anchoring exosomes to dECM networks, microparticles, or degradable microspheres can prolong their retention time at the defect site and reduce their rapid clearance from the body's fluid environment⁷⁸. For bone repair, exosomes are better suited as adjuncts to promote cell migration, angiogenesis, and local tissue remodeling, rather than being viewed as standalone solutions capable of replacing structural scaffold materials^{72,149}. Their clinical translation still requires addressing issues such as the selection of source cells, isolation and purification, dose determination, batch consistency, and storage conditions¹⁴⁹.

Applications of drug-loaded, expandable dECM constructs in infection, inflammation, and localized bone metabolic abnormalities¹³⁵. For infectious bone defects, antimicrobial drugs can be loaded into the material to maintain high local effective concentrations at the defect site while minimizing the systemic burden of medication^{150,151}; For defects with pronounced inflammatory responses, local anti-inflammatory modulation may be considered; for patients with insufficient bone mass or active bone resorption, the use of drugs that improve local bone metabolism may also be explored. Drug release can be regulated through microsphere encapsulation, inorganic particle adsorption, dynamic cross-linked networks, or multilayer structures¹⁴³. More functions in a bio-construct do not necessarily equate to better outcomes; excessive layering of drugs, cells, and multiple bioactive factors significantly increases the complexity of component interactions, quality control, and safety assessment³. Therefore, a “minimum sufficient design” should be adopted based on the clinical problem: for ordinary bone defects, the focus should be on enhancing support and osteogenesis; for defects with poor blood supply, emphasis should be placed on vascularization; and for infectious defects, priority should be given to addressing the temporal coordination between local antimicrobial action and subsequent regeneration.

From the perspective of stage-specific needs, the loading of bioactive substances primarily enhances cell recruitment, angiogenesis, and osteogenesis during the repair phase; however, the therapeutic window can be shifted earlier or later depending on the loaded components: bioactive

components with immunomodulatory effects can be used to improve the early inflammatory microenvironment, while sustained or time-controlled release of osteogenic and pro-angiogenic factors is better suited to support new tissue formation and subsequent maturation during the repair phase¹⁵².

5.5 Bionic Structural Design

Bionic structural design is a critical step in translating the advantages of dECM materials into actual tissue repair capabilities⁹⁴. Natural bone is not a homogeneous entity, but rather a multiscale system composed of cortical bone, cancellous bone, the medullary cavity, the periosteum, and a vascular network^{16,28}. Different regions perform distinct functions in force transmission, nutrient exchange, and bone regeneration¹⁵³. Therefore, biomimicry does not imply mechanically replicating the entire morphology of natural bone, but rather constructing a rational spatial environment in terms of macroscopic shape, mesoscale porosity, and microscale material distribution based on the defect site, load characteristics, and regenerative requirements.

At the macro level, customized contour design can improve the fit between the prosthesis and the margins of the bone defect. After creating a 3D model using CT or MRI data, the prosthesis's contour, contact surfaces, fixation areas, and implantation orientation can be pre-designed⁷⁷. For irregular bone defects, good contour matching helps reduce intraoperative trimming, minimize marginal gaps, and improve initial stability^{29,31}; for complex sites such as periarticular, pelvic, or maxillofacial regions, it also better restores local morphology and biomechanical relationships¹⁵⁴. Macro-level design should primarily serve surgical procedures, fixation methods, and structural safety, rather than merely pursuing a high degree of aesthetic fidelity.

At the mesoscale level, the pore structure determines the balance among cell infiltration, vascular ingrowth, and mechanical support²⁶. Connected pore channels facilitate nutrient transport, metabolite removal, and tissue ingrowth into the construct; however, excessive porosity weakens the material's strength, while pores that are too small restrict cell entry and vascular formation³⁰. Therefore, pore size, connectivity, pore orientation, and local porosity should be designed in conjunction with the material properties and the loading environment. For load-bearing regions, a relatively dense peripheral support zone and primary load-bearing pathways can be established; for internal regeneration zones, a more highly connected porous network can be employed.

Through a “peripheral support–internal regeneration” zoned design, a more reasonable balance can be achieved between early-stage stability and late-stage bone ingrowth¹⁵⁵.

At the microscopic level, local gradients can be created through variations in print paths, fiber orientation, inorganic component distribution, dECM microparticle enrichment, and the degree of cross-linking^{6,7}. In natural bone, collagen fibers and mineralized phases are not uniformly distributed; their directionality and gradients influence local mechanical force transmission and cellular behavior^{32,33}. Bioprinting enables the creation of regions with continuous transitions from high to low mineralization, from dense to porous, and from high to low stiffness¹⁰⁵. This strategy is particularly suitable for bone-soft tissue junctions, osteochondral interfaces, and the edges of repair sites, as it reduces stress discontinuities and interface delamination caused by the direct splicing of different materials.

Vascular biomimetic structures are particularly important for large-volume bone constructs. Relying solely on random porosity makes it difficult to ensure nutrient supply to the central regions of the construct, a problem that is especially pronounced when the material is thick or the cell density is high²². By incorporating regular channels, branched networks, or utilizing sacrificial materials to form through-channels, more defined pathways can be provided for perfusion culture, cell distribution, and post-implantation vascular ingrowth²⁶. Channel design must be coordinated with the overall scaffold structure to avoid significantly reducing the strength of the construct due to excessive permeability^{73,156}. In clinical applications, vascular biomimetic strategies must also be considered in conjunction with the host blood supply, soft tissue coverage, and fixation methods.

For the repair of osteochondral or bone–soft tissue interfaces, layered and gradient biomimicry is particularly critical⁴. The superficial layer must remain relatively soft, hydrated, and abrasion-resistant, while the deep layer should exhibit higher mineralization and load-bearing capacity; an intermediate zone must provide a continuous transition to prevent interface damage caused by abrupt changes in material properties⁷⁶. Through multi-material printing, zone-specific cross-linking, and regulation of mineralization levels, relatively continuous bone-cartilage-like structures can be formed within a single construct¹⁵⁷. Evaluation of such constructs should not be limited to short-term cell viability or single mechanical parameters; it should also include a comprehensive analysis of pore connectivity, the synchronization of material degradation with

new tissue formation, interface stability, *in vivo* vascularization, and the structure's ability to maintain its integrity under long-term loading.

Bionic structural design also plays a significant role across different stages: in the early post-implantation phase, matching the shape of the defect and the load-bearing path helps maintain spatial and mechanical stability; during the repair phase, interconnected pores, vascular channels, and gradient structures promote cell infiltration, nutrient exchange, and vascular ingrowth; and once the remodeling phase begins, pore evolution, material degradation, and interface continuity further influence new bone integration and long-term load transfer¹⁵⁸.

In summary, advanced strategies for dECM bioprinting should be based on systematic design. At the material level, the synergy between dECM, inorganic mineralized components, and stable scaffold frameworks is better suited to address both the bone-like microenvironment and early mechanical requirements; at the process level, mild yet thorough physical, chemical, and enzymatic treatments are prerequisites for ensuring material safety and functionality; at the functional level, the loading of bioactive substances should emphasize localization, low dosage, and time-controlled release¹⁵; At the structural level, personalized, porous, gradient, and vascular-mimetic designs determine whether the construct can truly adapt to complex bone defects¹⁵⁹. As a representative example of the aforementioned co-design approach, Kim et al. spatially arranged endothelial cell spheroids between printed BdECM/ β -TCP pillars loaded with human adipose-derived mesenchymal stem cells, and demonstrated the construct's comprehensive performance in terms of osteogenesis and vascularization through *in vitro* and *in vivo* evaluations⁷⁵. (Figure 6) Only by integrating these strategies into a unified design framework can dECM bioprinting evolve from material-level innovation into an evaluable, reproducible bone regeneration approach with potential for clinical translation^{118,159}.

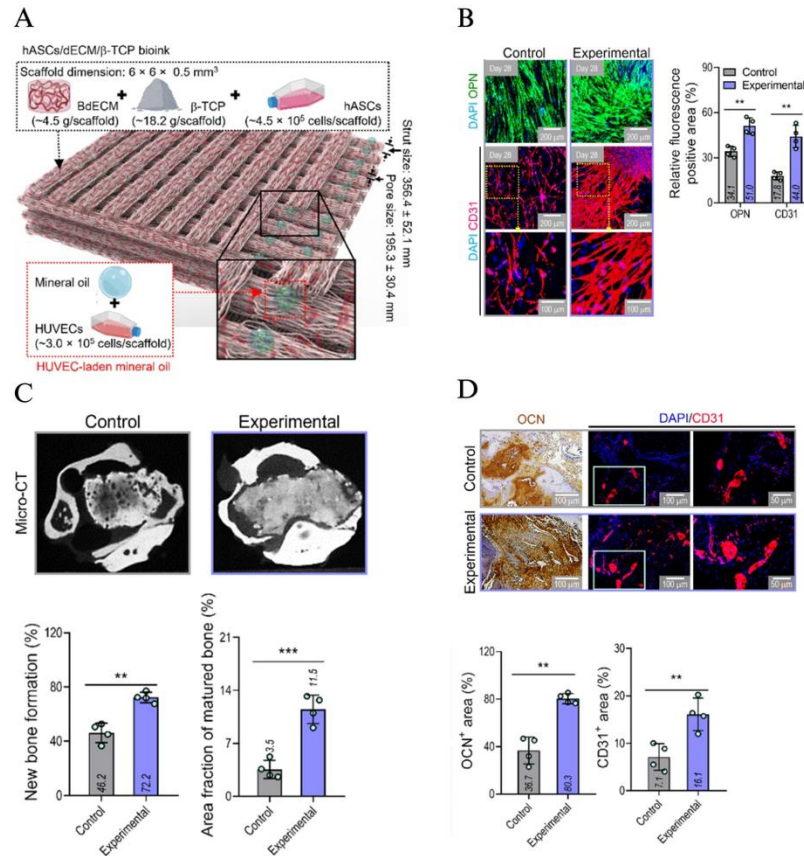


Figure 6. Spatial organization of endothelial spheroids in BdECM/β-TCP constructs promotes vascularized bone regeneration. (A) Schematic illustration of the hybrid construct in which endothelial cell spheroids were spatially positioned between hASC-laden BdECM/β-TCP struts. (B) Representative OPN and CD31 fluorescence images and corresponding quantitative analyses showing *in vitro* osteogenic and angiogenic responses. (C) Micro-computed tomography images at 8 weeks and quantitative analyses of newly formed and mature bone in the rat mastoid obliteration model. (D) Representative OCN and CD31 staining and corresponding quantitative analyses demonstrating osteogenic tissue formation and vascular development *in vivo*. Reprinted with permission from Kim W et al. ⁷⁵ Copyright ©2022, Ivyspring International Publisher.

6. Conclusion

The key to dECM bioprinting lies not in simply replicating natural bone tissue, but in constructing a regenerative microenvironment that simultaneously meets the requirements for early stability, cell-friendliness, vascularization, and long-term bone remodeling through the synergistic optimization of source selection, materials engineering, functionalization, and biomimetic structural design. For orthopedic issues such as large bone defects, osteochondral injuries, bone infections, and bone reconstruction following tumor resection, dECM bioprinting

holds promise as a complementary alternative to traditional bone grafts and artificial bone substitutes when combined with personalized imaging-guided design, localized delivery of bioactive substances, and multi-material construction. However, its clinical translation still faces challenges such as variations in dECM sources and batches, inconsistent decellularization and sterilization standards, insufficient mechanical support, difficulty in precisely controlling degradation behavior, safety concerns regarding active components, and difficulties in large-scale preparation. In the future, a standardized system covering raw material preparation, quality assessment, printing parameters, *in vivo* safety, and long-term functional outcomes should be established, and its efficacy should be validated in large-animal models that more closely mimic clinical load conditions. Only by achieving a precise match between material properties, structural design, and actual orthopedic needs can dECM bioprinting further develop into a bone regeneration strategy with reproducibility and clinical value.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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